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CLEAN VERSION OF PENDING CLAIMS

VIRUSES COMPRISING MUTANT ION CHANNEL PROTEIN

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1. (Amended) An isolated and purified recombinant influenza virus comprising a mutant ion channel protein which lacks or has reduced activity relative to the corresponding wild-type ion channel protein, wherein the mutation is in the transmembrane domain of the ion channel protein, and wherein the mutation does not alter the *in vitro* replication of the virus but is associated with attenuation of the virus *in vivo*.

2. (Amended) An isolated and purified recombinant influenza virus comprising a mutant ion channel protein which is a chimeric protein.

3. The isolated and purified virus of claim 1 wherein the mutant ion channel protein comprises at least one amino acid substitution.

4. (Amended) The isolated and purified virus of claim 3 wherein the substitution is in the transmembrane domain of the ion channel protein.

5. The isolated and purified virus of claim 1 wherein the mutant ion channel protein comprises a deletion.

6. The isolated and purified virus of claim 1 wherein the ion channel protein is the M2 protein of influenza A virus.

7. The isolated and purified virus of claim 1 wherein the ion channel protein is the NB protein of influenza B virus.

8. The isolated and purified virus of claim 1 wherein the ion channel protein is the CM1 protein of influenza C virus.
9. The isolated and purified virus of claim 1 wherein the recombinant virus further comprises a heterologous immunogenic protein of a pathogen.
10. A vaccine comprising the isolated and purified virus of claim 1.
11. A method of preparing a recombinant influenza virus comprising a mutant ion channel protein which lacks or has reduced activity relative to the corresponding wild-type ion channel protein, comprising:
 - (i) contacting a host cell with a plurality of influenza vectors so as to yield recombinant influenza virus, wherein the plurality of vectors comprises: a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises mutant ion channel protein DNA; and b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a

vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2; and

(ii) isolating the virus.

12. The method of claim 11 wherein the mutant ion channel protein is a mutant influenza virus ion channel protein.

13. The method of claim 11 wherein the mutant ion channel protein is an influenza virus A ion channel protein.

14. A vector encoding a chimeric protein comprising the ectodomain of an influenza virus ion channel protein linked to a transmembrane domain of a heterologous protein linked to a cytoplasmic domain that is not the cytoplasmic domain of hepatitis B core.

15. The vector of claim 14 wherein the heterologous transmembrane domain is from a protein that is not an ion channel protein.

16. The vector of claim 14 wherein the cytoplasmic domain is that of an influenza virus ion channel protein.

17. The vector of claim 16 wherein the heterologous transmembrane domain is from an influenza virus protein.

18. A method to immunize a vertebrate, comprising: contacting the vertebrate with an effective amount of the recombinant virus of claim 1.

19. The method of claim 18 wherein the vertebrate is an avian.

20. The method of claim 18 wherein the vertebrate is a mammal.

21. The method of claim 18 wherein the vertebrate is a human.

22. A composition comprising a plurality of influenza vectors, comprising:

a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises a mutant ion channel protein DNA; and

b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a

DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

23. The composition of claim 22 further comprising a vector comprising a promoter operably linked to a DNA fragment of interest in antisense orientation.

24. The composition of claim 23 wherein the vector comprises a DNA fragment which encodes an immunogenic polypeptide or peptide of a pathogen.

25. (Amended) An isolated virus prepared by contacting a host cell with a plurality of influenza vectors, wherein the plurality of vectors comprises: a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an

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influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises mutant ion channel protein DNA comprising a mutation in the transmembrane domain which mutation does not alter the *in vitro* replication of the virus but is associated with attenuation of the virus *in vivo*; and b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

26. A host cell contacted with the virus of claim 1 or 25.

27. (New) The isolated and purified virus of claim 2 wherein the chimeric protein comprises the N-terminal and C-terminal portion of an influenza virus ion channel protein and the transmembrane domain of a heterologous protein.

28. (New) The isolated and purified virus of claim 27 wherein the chimeric protein comprises an influenza virus ion channel protein and the transmembrane domain of a hemagglutinin or neuraminidase protein.

29. (New) The isolated and purified virus of claim 3 wherein the substitution is at a residue corresponding to residue 27, 30, 31, 34, 38 or 41 of the transmembrane domain of M2.

30. (New) The isolated and purified virus of claim 29 wherein the substitution is a threonine for valine at residue 27, a proline for alanine at residue 30, an asparagine for serine at residue 31, or an alanine for tryptophan at residue 41.
31. (New) The isolated and purified virus of claim 5 wherein the deletion includes residues corresponding to residues 29 to 31 of the transmembrane domain of M2.